Isolation and Characterization of 17 Polymorphic Microsatellite Loci for *Musella lasiocarpa* (Musaceae)

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Additional index words. Chinese dwarf banana, golden lotus banana, molecular markers, population genetics

Abstract. Seventeen microsatellite markers were isolated from 24 wild individuals collected from three populations of *Musella lasiocarpa* (Franch.) C. Y. Wu ex H. W. Li, a plant endemic to southwest China. The average allele number of these markers was 3.6 per locus, ranging from two to seven. The expected (H_e) and observed (H_o) heterozygosities varied from 0.4672 to 0.8183 and from 0.0417 to 1, respectively. Eleven loci were significantly deviated from Hardy-Weinberg equilibrium as a result of the heterozygote deficiency. These microsatellite primers will be used for studying genetic population structure and genetic diversity of *M. lasiocarpa*.

Musella lasiocarpa, commonly referred to as Chinese dwarf banana or golden lotus banana, is native to the watersheds of the Upper Yangtze River of Yunnan and Sichuan provinces in southwest China (Wu and Kress, 2000). It is a synonym of *Musa lasiocarpa* Franch. (Cheesman, 1947) and *Ensete lasiocarpum* (Franch.) E. E. Cheesman (Simmonds, 1960). Different from the triploids with various combinations of A and B genomes of *Musa*, *Musella lasiocarpa* is diploid with a chromosome number $2n = 18$ (Isobe and Hashimoto, 1994). With its showy golden yellow flowers, which can last up to 7 months (Liu et al., 2003), and abroad ecological environment adaptability, this species has attracted some horticulturists from the United States, the United Kingdom, European Union, and Japan, who have taken an interest in the commercial development of this resource as

an ornamental (Long et al., 2008). Aside from the decorative value, the use of *M. lasiocarpa* has also been reported as medicine, wine-brewing, weaving material, source plant for honey, pig fodder, and as valuable for soil and water erosion control (Liu et al., 2003; Long, 1997; Long et al., 2008). Although this plant has been introduced to various regions and botanical gardens and has been cultivated widely in Yunnan, the wild populations of *M. lasiocarpa* are rare as a result of highly fragmented habitats (Liu et al., 2002). To provide effective conservation and management, better understanding of the wild population genetic structure of this species is important. In this study, we reported the characterization of 17 polymorphic microsatellite loci to investigate genetic structure among populations of *M. lasiocarpa* for further conservation genetic studies.

Materials and Methods

Twenty-four *Musella lasiocarpa* individuals from three populations were used in our study (Table 1). Genomic DNA samples were extracted using the cetyltrimethylammonium bromide extraction method (Milligan, 1992). The isolation method of loci was performed according to the fast isolation by amplified fragment length polymorphism of sequences containing repeats (Zane et al., 2002). Briefly, ≈ 300 ng genomic DNA was digested

with a restriction enzyme-*MseI* (Fermentas) and ligated to an *MseI* adaptor pair (Vos et al., 1995); then 5 μ L of ligation products acted as templates to perform polymerase chain reaction (PCR) in a volume of 20 μ L using *MseI*-N primers (5'-GAT GAG TCC TGA GTA AN-3') and following the program: 95 °C for 3 min, 30 cycles of 94 °C for 30 s, 53 °C for 60 s, 72 °C for 60 s, followed by 72 °C for 5 min.

For enrichment, the PCR products were denatured at 95 °C for 5 min and then were hybridized with 5'-biotinylated probe (AG)₁₅ in a 250 μ L solution (20 $\times$ SSC, 10% SDS, 100 pmol/ μ L) at 48 °C for 2 h. The DNA hybridized to the probe was separated and captured by streptavidin-coated magnetic beads at room temperature for 20 min followed by two washing steps, including three times in TEN₁₀₀ for 15 min and three times in TEN₁₀₀₀ for 24 min. The separated single-stranded DNA was subjected to a second round of PCR according to the same procedure as the first round of PCR. The PCR products, purified with the E.Z.N.A Gel Extraction Kit (Omega Bio-Tek, USA), were ligated into PMD18-T vector (Takara, Japan) according to the manufacturer's instructions and then transformed into *Escherichia coli* strain JM109 (Sangon, China). The positive clone was picked out by blue-white screening and tested by PCR using (AG)₁₀ and M13⁺/M13⁻ as primers, respectively. One hundred fifty-five of 277 screened clones contained potential microsatellite motifs.

A total of 100 clones (67%) were found to contain simple sequence repeat and then subjected to primer designing using the Primer 5.0 (Clarke and Gorley, 2001). Twenty-four *M. lasiocarpa* individuals from three wild populations were used to screen polymorphism. PCR reaction was done in 20 μ L volume using PTC200 thermal cycler (MJ Research, USA). Each reaction was performed using 20 ng of genomic DNA, 0.1 mM of each dNTP, 1 μ M each primer, 1 $\times$ Taq buffer [100 mM Tris-HCl, pH 8.0, 2.0 mM MgCl₂, 200 mM (NH₄)₂SO₄, 0.1% Tween 20] and 1 U of Taq polymerase (Takara). The PCR programs took place as follows: initial denaturing step at 95 °C for 5 min, 30 cycles of 94 °C for 30 s, primer-specific annealing temperature 55 to 62 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 8 min. The PCR products were electrophoresized in denaturing 6% polyacrylamide gels using a 25-bp DNA ladder molecular size standard (Fermentas) to estimate allele sizes by silver staining.

Results and Discussion

Of these 42 new primers designed for *M. lasiocarpa*, 28 successfully amplified the target regions and 17 of them displayed polymorphism. Results of the allele number, expected (H_e) and observed (H_o) heterozygosities and Hardy-Weinberg equilibrium (P) were calculated by GENETOP version 3.4 (<http://genetop.curtin.edu.au/>) (Raymond and Rousset, 1995). The number of alleles per locus ranged from two to seven (Table 2). The

Received for publication 25 June 2009. Accepted for publication 10 Sept. 2009.

This work was supported by the Ministry of Science and Technology of China (2008FY110400-2-2 and 2005DK21006), the Ministry of Education of China (B08044 and CUN-985-3-3), and the Japan Society for the Promotion of Science (JSPS/AP/109080).

Dr. Anjun Tang and Yitao Liu made contributions in collecting samples from the fields.

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Table 1. Sample information of *Musella lasiocarpa*.

Species	Distribution	Habitat	Altitude (m)	Position	Sample size
<i>Musella lasiocarpa</i>	Bingju, Dali, Yunnan Province	Limestone mountain	1795	Lat. E 100°30'59.7", long. N 25°40'38.1"	8
<i>Musella lasiocarpa</i>	Jingjiang, Lijiang, Yunnan Province	Steep mountain beside Jingsha River xerothermic valley	1870	Lat. E 100°25'57.0", long. N 26°58'58.0"	8
<i>Musella lasiocarpa</i>	Qinhe, Yanyuan, Sichuan Province	Steep mountain beside Jingsha River xerothermic valley	1400	Lat. E 101°24'07.2", long. N 27°04'39.5"	8

Table 2. Primer sequences, repeat motif, size range, annealing temperature (T_a), number of alleles (A), expected (H_e) and observed (H_o) heterozygosities, and significance of deviation from Hardy-Weinberg equilibrium ($P < 0.01$) for 17 microsatellite primers from *Musella lasiocarpa*.

Locus	GenBank accession no.	Primer sequence (5'-3')	Repeat motif	Allele size (bp)	T_a (°C)	A	H_e	H_o	HWE (P value)
B1	GQ144662	R GGGTTTTGTGGGTTATTTGA F ACCTTCTTTGGGAACATATGG	(CT) ₁₁	181–188	54	5	0.7917	1.0000	0.0114
B2	GQ144663	R GAGATCCTTCTTTAGTTCCA F ATGTCTTGCTTGTCCTGAG	(TC) ₁₁	311–317	54	3	0.5098	0.5000	0.8093
B4	GQ144664	R ATCTACCACAATGGCTGACA F AAGCTTCTAACTCTTTCTGC	(CT) ₅ (CT) ₆	153–157	58	3	0.5629	0.3333	0.0020*
B5	GQ144665	R TCAAAGGGAAGAGGGAACAG F GACTAAGCCCAACCCAAAT	(GA) ₉	247–251	61	3	0.6569	0.9167	0.0094*
B8	GQ144666	R TACAAGCAAGCAAAAGCGAC F CACCAAGGTGCAATATCAAG	(CA) ₇ (CT) ₈	176–182	59	3	0.6800	0.4583	0.1013
B9	GQ144667	R AACTCACAGGACCTAACAAAG F TACAGAAACATCGGTATCAT	(AC) ₉ (AG) ₆	324–328	55	3	0.4814	0.0417	0*
B11	GQ144668	R GTGGTGGTGATGGCGAATAG F AGGATGACTGCGACTTGACG	(CT) ₁₁	160–168	11	4	0.7021	0.0833	0*
B16	GQ144669	R AGATCTGGCAGTGAATGGT F TGTTCCTGCTCAATAGA	(GA) ₁₅	227–231	60	3	0.6241	0.2500	0*
B17	GQ144670	R CTTCTTCCTCATCCCTCGTT F ATTGCCCTCTTGACTCCTTT	(CT) ₁₆ (CA) ₆	188–192	54	3	0.6250	0.5417	0.0001*
B20	GQ144671	R ATGTAGACAAAAGCTCCAGTT F CAAATACAAGCCTAAATCTC	(AG) ₁₅	365–381	54	5	0.5816	0.2500	0*
B23	GQ144672	R ATTGCCCTCTTGACTCCTTT F CTTCTTCCTCATCCCTCGTT	(TG) ₆ (AG) ₁₅	186–190	58	3	0.6108	0.5417	0.0001*
B24	GQ144673	R CACCAAGGTGCAATATCAAG F GAATGATTTGCTGAGCGAAG	(GA) ₁₀	152–166	60	7	0.8183	0.5000	0*
B25	GQ144674	R CAGACACCGGCTCAAACAAG F ATCAGACGGCGATGAAAGAA	(CT) ₉	226–230	56	2	0.4965	0.7500	0.0154
B28	GQ144675	R CAAGAAGAAAAGATCATCGC F GACTTGACACGTCGTGAATT	(CT) ₁₀	200–205	60	4	0.7332	0.7083	0.0019*
B34	GQ144676	R TTGAAATGGAATGGGTCTG F TCTGTGAGTTACATGGTGGG	(CT) ₉	180–186	60	4	0.7367	0.3333	0*
B35	GQ144677	R ACAACCGTTCTGTGACAAT F GAAGGCTACGTCCAACAC	(CT) ₉	157–167	60	4	0.7012	0.7500	0.0403
B37	GQ144678	R TACCTTGCACCTTTTCTTCT F ATCATTTCAAGCAAACAGTG	(CT) ₈	222–224	60	2	0.4672	0.3750	0.3921

*Significant deviation from Hardy-Weinberg equilibrium (HWE) ($P < 0.01$).

expected (H_e) and observed (H_o) heterozygosities ranged from 0.4672 to 0.8183 and 0.0417 to 1, respectively. Among 17 microsatellite markers, 11 loci showed significant deviation from Hardy-Weinberg equilibrium ($P < 0.01$), probably due to deficiency of heterozygote or the limitation of sample size. Tests for linkage disequilibrium were run in FSTAT Version 2.9.3.2 (Goudet, 1995). Significance levels were adjusted using sequential Bonferroni corrections (Rice, 1989). No loci showed significant linkage disequilibrium after Bonferroni correction.

The polymorphic microsatellite loci presented here would be useful for studying the population genetic structure and genetic diversity of *M. lasiocarpa*, which would help us to better understand the wild *Musella* population and then to provide effective conservation and management.

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